EXHIBIT M

Documenta Ophthalmologica 61, 303–312 (1986).

Mechanical and biochemical effects of man-made fibres and metals in the human eye, a SEM-study

W.L. JONGEBLOED[1], M.J. FIGUERAS[2], D. HUMALDA[3], L.J. BLANKSMA[4] and J.G.F. WORST[5]

[1] Centre for Medical Electronmicroscopy and [3] Laboratory for Histology, University of Groningen; 69/72 Oostersingel, 9713 EZ Groningen, The Netherlands
[2] Department of Biology, Faculty of Medicine, University of Barcelona, Reus, Spain
[4] University Eyeclinic, Groningen and [5] Eye Physician and Surgeon, Julianalaan 11, Haren, The Netherlands

Key words: biodegradation, mechanical damage, surface roughness, prolene, perlon, supramid, titanium

Abstract. Prolene, perlon, supramid and titanium, either used as sutures, 'Strampelli' sutures or artificial lens loops, were compared after remaining in the human eye for at least one year. For comparison, non-implanted samples of each of the materials were used as blanks. Prolene and Perlon in particular showed severe biodegradation after a given period; Supramid showed much less degradation of its surface. Titanium showed a rather rough outer surface (even in the non-implanted samples), particularly as bends in the lens loop, which facilitated the adherence of cells, fibres and microorganisms.

Introduction

Prolene, perlon and supramid are widely used as suture material for lens haptic material in the human eye. The biocompatibility of these materials has been the subject of investigation by several authors: Hessburg (1985), Apple et al. (1984), Drews (1983a), Drews (1983b), Olson (1979) and Drews et al. (1978).

Nevertheless there is still no clear explanation of the phenomenon one can observe in the pictures, although there are strong indications for enzymatic action being the cause of the surface changes on these materials. On the other hand all kinds of explanations are given, such as: fixation or drying artifacts, mechanical damage when the lens and loops are taken out of the eye, damage caused by the irradiation in the SEM, possible effects of the sterilization agents, UV-light etc.

To exclude differences in handling and preparation for the SEM, a uniform preparation method was used for all materials, while for the controls so-called surgery-made material was used.

Materials and methods

The prolene material was part of an open J-lens, which had remained in the eye of a Pakistani patient (sample provided by Dr Cristie) for 1 year. The lens had been placed in the chamber-angle, causing progressive endothelial damage

and corneal oedema, and was removed for that reason. The perlon 'Strampelli' suture material had been in place in a Dutch patient for 6½ years and was removed because the suture had broken at both ends. The supramid lens loop of a Fedorov-type lens was removed from a Dutch patient after 10 years for surgical reasons. The titanium lens loop and lens of a Dutch patient were removed, after remaining 3½ years in the eye, also for surgical reasons.

All samples, including the non-implanted ones, were air-dried and subsequently sputtercoated with gold (appr. 150 nm) and examined in a JEOL-35C-type SEM, operated at 15 kV.

Results

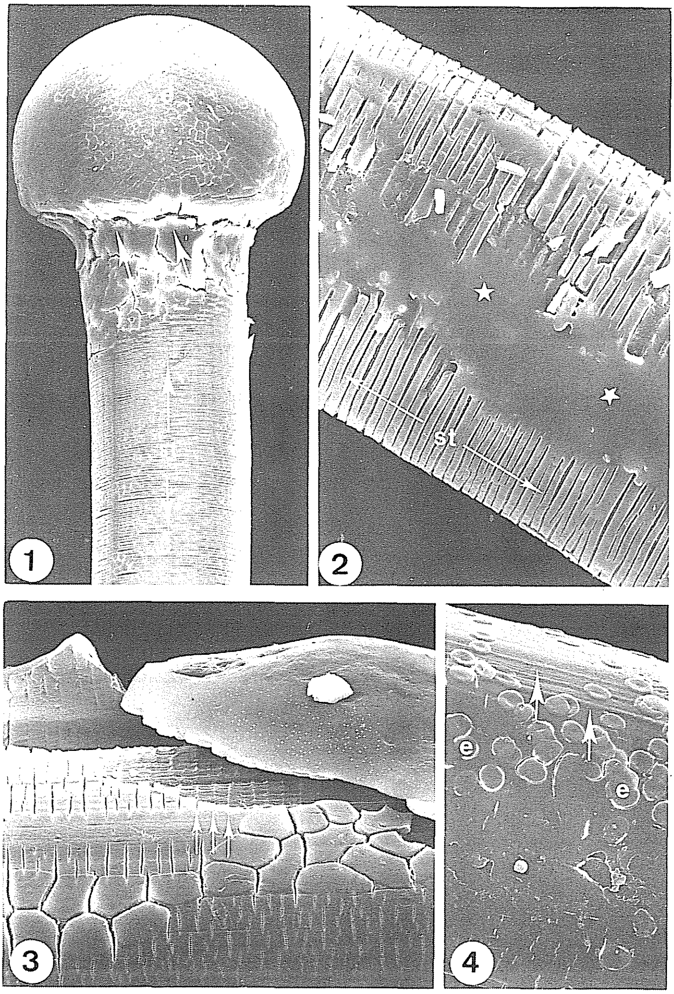
Prolene

Both prolene loops showed severe degradation of the surface layer. Figure 1 shows part of one of the loops with a very regular pattern of striations (st) perpendicular to the longitudinal axis of the loop. The rounded end of the loop and the beginning of the straight part of the loop are covered with a rather thick layer of deposits of an unknown substance which contains many erythrocytes (e). The irregular cracks in this deposit (see arrows) are due to drying, a common phenomenon in SEM-specimens of inhomogenous structure. Figure 2 shows at higher magnification the very regular pattern of striations (st); some parts of the surface are covered with a thin deposit (*), comparable to the layer seen in Figure 1. The degradation of the surface layer can be observed in more detail in Figure 3. Part of the surface layer is detached, exposing the *sub*-surface layer, which shows a similar cracking pattern (see arrows). Although the cracking can have been enhanced by the drying process, the loops and lens were dried in air, the fact that the sub-surface layer shows a similar cracking pattern of degradation is a clear indication of biodegradation. If the phenomenon we observe were the result of the drying of a deposit, then the cracks in it would have a much more irregular character, like the cracks seen in Figure 1 on the rounded top. Moreover the total diameter of the degraded loop has certainly not increased, which would be the case if the result we observe was caused by the deposition of material.

That part of the loop (close to the lens) which was not in contact with tissue did not show the degradation phenomenon, but was merely covered with a very thin deposit, consisting mainly of erythrocytes (e), Figure 4. The layer of deposit is very thin, as can be concluded from the longitudinal lines, caused by the manufacturing process, which are still visible despite the deposit (see arrows).

Perlon

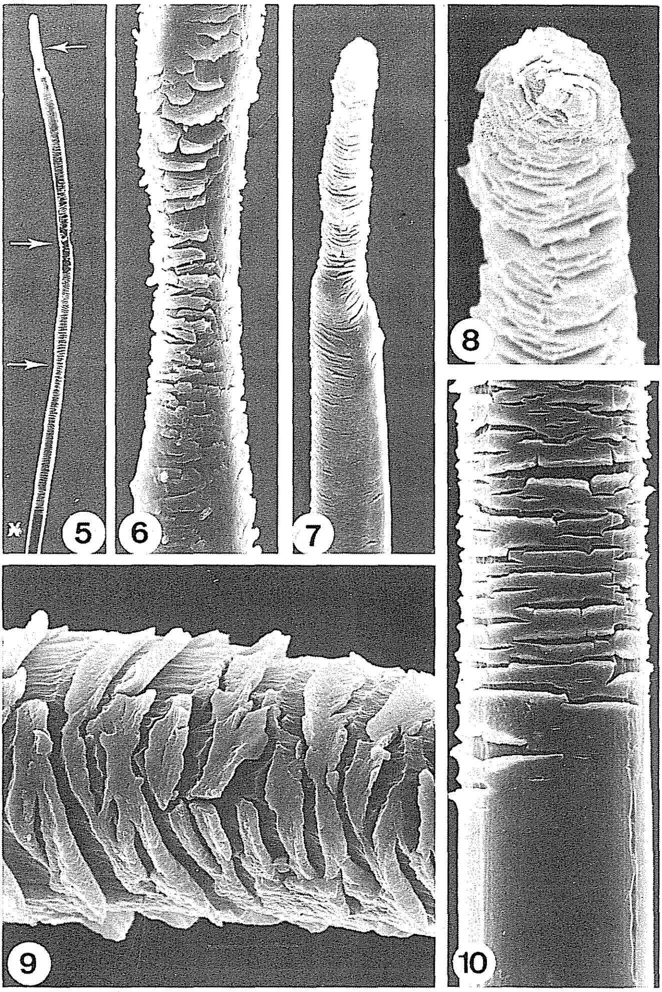
The perlon 'Strampelli' sutures also showed severe degradation at the ends which had been in contact with the tissue, the middle part of the suture did



Figures 1–4. Prolene: Figure 1 (155 ×), part of prolene loop; e = erythrocyte covering; st = striations of biodegradation; arrows denote deposited organic matter. Figure 2 (370 ×), degraded surface; * = deposited organic matter; st = regular pattern of striations. Figure 3 (770 ×), detail of degraded surface layer. Figure 4 (370 ×), non-degraded prolene surface; e = erythrocytes; arrows denote manufacturing grooves.

not show this. The attack was so serious that the ends became so thin that they lost their strength and broke off. Figure 5 shows the broken end of one of the sutures at low magnification. In several places (see arrows) the suture had a smaller diameter than average. This considerable decrease in diameter is something we did not observe with prolene. The main part of the suture in Figure 5 shows a degraded surface, except for the part indicated by (*).

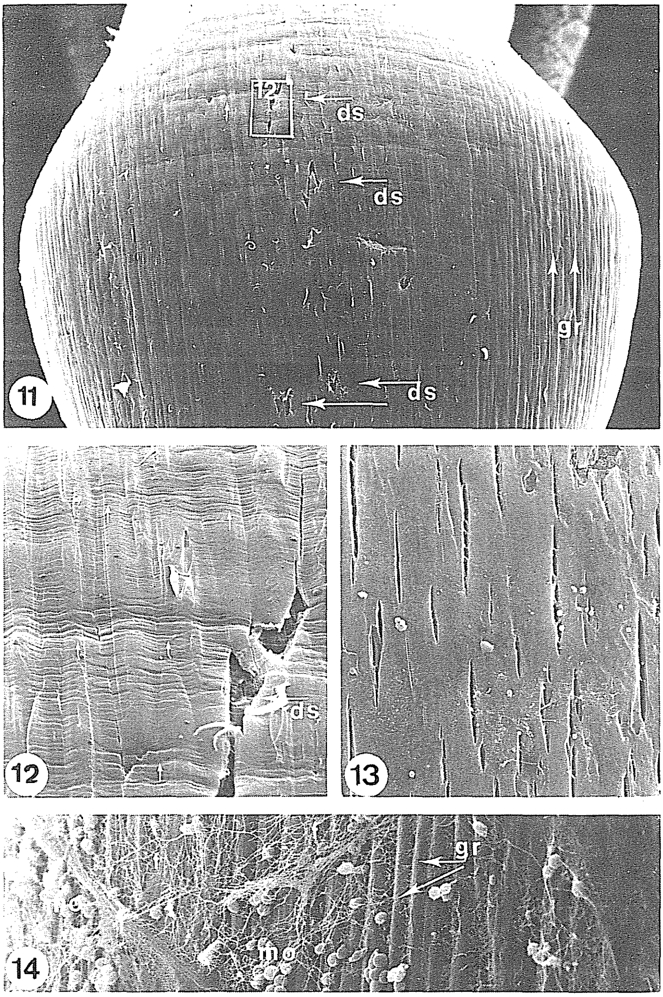
A thinner part of the suture is shown in more detail in Figure 6, the



Figures 5–10. Perlon: Figure 5 (38 ×), low magn. broken Strampelli suture; arrows denote decreased diameter; * = non-degraded part. Figure 6 (390 ×), detail of suture with decreased diameter. Figure 7 (175 ×), broken end of suture. Figure 8 (595 ×), detail of Figure 7. Figure 9 (1330 ×), detail of degraded surface. Figure 10 (450 ×), transition non-degraded (lower part) – degraded surface.

surface of the suture is degraded. The broken end of the suture is depicted in Figure 7, it has a considerably decreased diameter and the surface is degraded. The broken end is shown in Figure 8 at high magnification, note the rough outer surface.

Although not exactly the same, the general pattern of degradation of the surface layers of perlon (Figure 9) and prolene (Figure 2) is comparable. A similar pattern is found on the sub-surface layer of perlon to that seen in



Figures 11–14. Supramid: Figure 11 (455 ×), bend in loop; gr = manufacturing grooves; pl = lines perpendicular to grooves; ds = surface damage. Figure 12 (1530 ×), detail of Figure 11. Figure 13 (950 ×), longitudinal cracks in surface. Figure 14 (1530 ×), gr = manufacturing groove; f = fibres; mo = microorganisms.

prolene. That part of the suture which had not been in contact with tissue did not show these effects of biodegradation. Figure 10 shows such a transition area in more detail, the lower part of the picture represents the non-degraded surface of the perlon structure.

The pictures shown represent only part of the suture: the other end of the suture showed a similar pattern of degradation, only the middle part of the suture was not degraded.

Supramid

The supramid material showed, in particular at bends in the loop, a fine network of lines (see arrows) perpendicular to the longitudinal manufacturing grooves (gr) of the loop (Figure 11). On the median part of the loop, clear evidence of surface layer damage can be seen (ds). Figure 12 shows a detail of such a site and its relationship to the fine network already mentioned. In Figure 13 longitudinal cracks are visible on the surface of the supramid loop, possibly the start of biodegradation. These cracks are found in the vicinity of bends in the loop at some distance from them, implying that this type of degradation is related to weak spots in the loop, particularly bends due to the manufacturing process. The general pattern of the surface is not one of severe degradation, as compared to the degradation seen in prolene and perlon, at least not in the loops we investigated.

The relatively rough outer surface of supramid, as compared with prolene and perlon, certainly facilitates the adherence of fibres, cells and microorganisms, as can be seen in Figure 14. The picture shows the almost complete diameter of the loop with fibres (f), possibly fibrin, and microorganisms (mo) covering the manufacturing grooves (gr) of the supramid.

Titanium

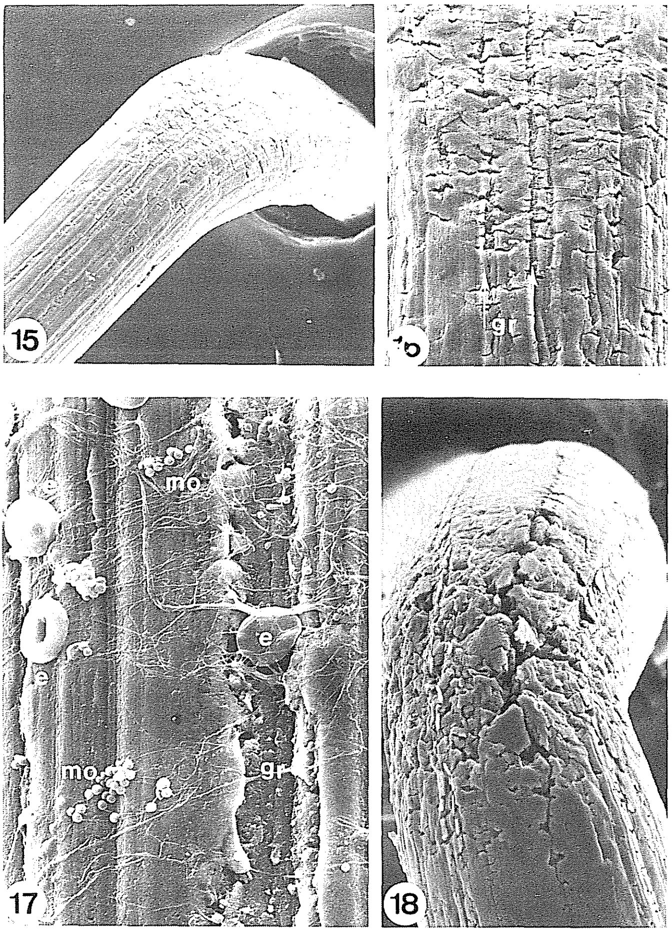
Titanium showed a rough outer surface, particularly at bends in the loop; in the straight part of the loop the longitudinal lines can be seen caused by the manufacturing process (Figure 15). The longitudinal grooves (gr) and the cracks more or less penpendicular to them can be seen in more detail in Figure 16 (area (A) of Figure 15). The roughness of the titanium surface facilitates the adherence of microorganisms (mo), fibre-like material and cells, especially erythrocytes (e), visible against the background of manufacturing grooves (gr) in Figure 17.

This roughness is already present in non-implanted material, as can be observed in Figure 18. The cracks in the surface layer particularly at the bends in the loop, visible in non-implanted as well as implanted material (see Figure 16), are some kind of mechanical degradation probably caused by, or at least initiated by, the manufacturing process.

This roughness also contributes considerably to irritation and inflammation of the tissue which is in contact with this material. In comparison with titanium, stainless steel-vanadium and, in particular, platinum wire have a much smoother surface.

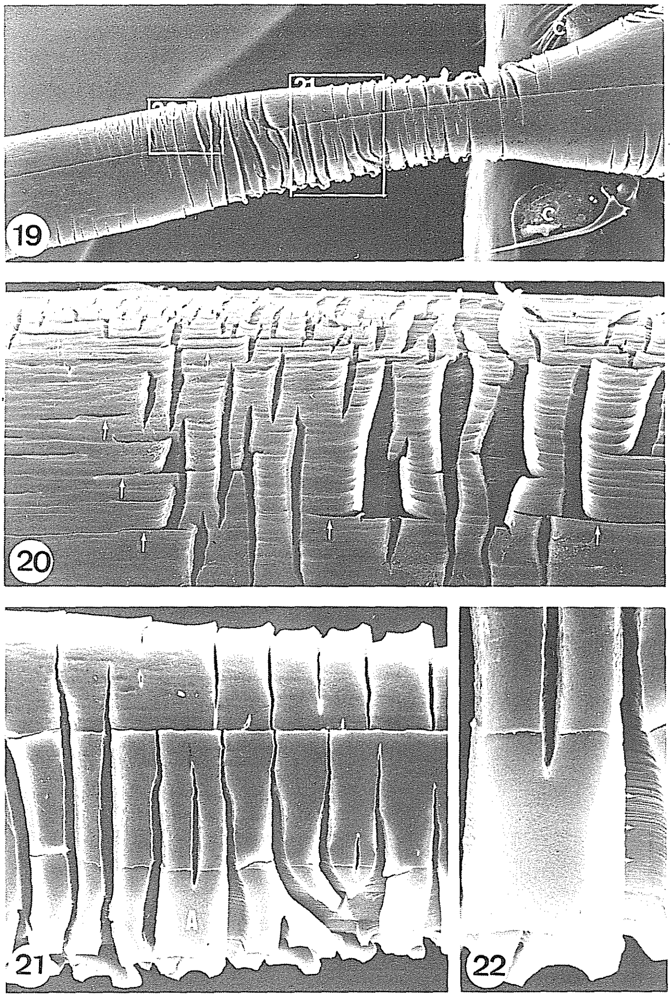
Addendum

Very recently we investigated another Fedorov-type IOL with 4 supramid loops, which had remained for several years in the eye of a Dutch patient. The loops did not show any biodegradation, *except* for a small part of



Figures 15–18. Titanium: Figure 15 (190 ×), bend in implanted loop. Figure 16 (455 ×), detail of surface of bend; gr = grooves. Figure 17 (1230 ×), detail of loop surface; mo = microorganisms; e = erythrocytes; gr = manufacturing groove. Figure 18 (345 ×), detail of bend in non-implanted loop.

one loop situated close to the IOL (see Figure 19), which showed severe biodegradation. The diameter of the loop had decreased at this site. The IOL showed some cell deposits around the loop 'entrance' (c). The degradation pattern is quite similar to that found in prolene and perlon (see Figures 20 and 21, both details of Figure 19). From Figure 20 it seems logical to assume that the degradation is connected with the surface morphology of the supramid surface. The longitudinal cracks (see arrows) are comparable with those found in Figure 13, which can be related to the manufacturing grooves. The



Figures 19–22; Supramid: Figure 19 (75 ×), part of loop close to IOL; c = cell deposit. Figure 20 (455 ×), detail of Figure 19; arrows denote longitudinal cracks. Figure 21 (275 ×). detail of Figure 19; severe degradation of surface. Figure 22 (610 ×), detail of Figure 21, inside of crack.

striations perpendicular to these cracks are comparable with the striations found in prolene (Figure 2) and perlon (Figure 9), although less regular.

Figure 22 shows more detail of the inner part of the surface layer (enlargement of part of Figure 21). From this picture we can conclude that the loosened surface layer does *not* have an amorphous structure, which would be the case if it was merely a deposit of, for instance, proteinic material. Arrows denote the cross-section of the loosened surface layer.

Discussion/Conclusion

The phenomenon observed on the surface of implanted PROLENE is a topic of interest to several authors, as already noted. The prolene we investigated had remained in the eye of a Pakistani patient for 1 year and showed severe changes on the surface of both loops. The samples were dried in air before sputtering with gold and examination in the SEM. Non-implanted material (which was already dry) given the same treatment, did not show any surface changes when examined in the SEM. Non-implanted material soaked in distilled water for a long time, dried in air, sputtercoated with gold and examined in the SEM under the same conditions, did not show any surface changes either. Inhomogenous material is known to show drying artifacts like cracks on the surface (both on drying in air and on critical point drying) when examined with the SEM. The cracks on the surface due to drying are always very irregular, comparable to the drying of clay in the sun.

We think that the surface changes observed in the implanted prolene are the result of biodegradation by the enzymatic action of the tissue fluids. Although the cracking pattern on the surface may be enhanced by the drying, the changes are the primary result of chemical changes on the surface.

This conclusion is supported by the images of the *sub*-surface layer, where lines appear perpendicular to the longitudinal axis of the loop, which are not present in the control sample. In some places a deposit is visible on top of the surface layer of the implanted prolene; this layer is very thin and not in any way comparable with the attacked layer. If the phenomenon of what we call biodegradation is the result of a deposit of, for example, a proteinic material, a quantitative increase in the average diameter would be the result. The total diameter at such sites did not increase but, on the contrary, tended to decrease slightly. Moreover the cracks (striations) are so regularly spaced over a large part of the implanted loops that they must be the result of prolene degradation and not of the mere drying of a deposit.

The effects seen in the perlon material are also the result of biodegradation; in certain places the material got so thin that it lost its strength and broke. Supramid material showed minor degradation effects, longitudinal cracks which may be related to the manufacturing grooves to some extent. In one case (see Addendum) we found, in a very small portion of the total length of all the loops, a similar degradation effect to that seen in prolene and perlon. Thus supramid can undergo biodegradation as well.

This brings us to two other considerations in connection with the phenomena shown: the influence of sterilizing agents and the necessary conditions for safe use of the materials.

Not much is known about the influence of sterilizing agents on the minute structure of the materials under consideration. Although sterilized non-implanted material does now show biodegradation, it is not known to what extent sterilization makes the man-made fibres more vulnerable to chemical

attack by enzymes, for example. Furthermore it is obvious that the site at which the material is being used influences the presence or absence of biodegradation. More research on these two factors needs to be done. Titanium did not show any surface changes after implantation for the given period.

Both supramid and titanium have initially a rather rough outer surface, which facilitates adherence and, in the case of titanium, contributes to irritation and inflammation of the tissue with which it is in contact.

References

Apple J, Mamalis N, Bradley SE, Loftfield K, Kavka-van Norman D and Olson RJ (1984) Biocompatibility of implant materials; A review and scanning electronmicroscopic study. Amer Intraocular Implant Soc J 10:53–64

Drews RC and Smith ME (1978) Scanning electronmicroscopy of intraocular lenses. Ophthalmology 85:415–423

Drews RC (1983) Quality control and changing indications for lens implantations. The Seventh Binkhorst Medal Lecture 1982. Ophthalmology 90:301–310

Drews RC (1983) Polypropylene in the human eye. Amer Intraocular Implant Soc J 9:137–142

Hessburg PhC (1985) Evidence still supports polypropylene haptics. Ocular Surg News 3/5:1

Olson RJ (1979) Intraocular lens quality, update 1979. Amer Intra-ocular Implant Soc J 6:16–17